Comparison of peptide separation methods to maximize the mutational landscape in a cell line model system used for neoantigen discovery

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Introduction

Currently, there is great interest in the potential of personalized cancer therapies. One of these approaches, the use of MHC peptides to reawaken a patient's own killing mechanisms, has gained renewed strength due to the availability of patient specific databases. Here we used WT and p53 NULL cells as a way to easily generate mutated peptides as surrogates for neoantigens. These cell lines were profiled by shotgun proteomics using two different separations online with an Orbitrap: 1) a standard nanoLC packed column and 2) a pillar-arrayed-column (µPAC). The data was used to develop an informatic proteogenomic pipeline, which could be applied to neoantigen discovery, and separation performance of the two methods compared for ability to detect mutated peptides.

Materials and methods

A nanoRSLC UltiMate 3000 coupled to an Orbitrap Q-Exactive Biopharma was used. Sample was loaded onto the 300µmIDx 5mm PepMap C18 trap column and separated either on a µPAC (Pillar-Arrayed-Column, PharmaFluidics) cartridge with 2µm interpillar distance and 2m separation path operated or on a PepMap C18 (2µm, 100Å; 75µm ID x 50cm). Data acquisition used a data-dependent process. Database search was conducted using a custom RNAseq derived database in Mascot v2.6.0 with following settings carbamidomethyl was set as fixed modification and trypsin was set as a protease.

Results

A comparison of mutated peptide ID on µPAC and PepMap columns in A375 cell line with WT p53 and CRISPR on p53. Comparison shows that separation on µPAC is more effective leading to higher mutated peptide IDs and higher protein sequence coverage which is important for neoantigen discovery. A script inspecting peptide coverage in a candidate proteins with mutated position was used to determine overall mutant peptide ID. Together 16848 (A375 NULL) and 18832 (A375 WT) peptides identified in PepMap nanoLC LC-MS/MS assay and 33657 (A375 NULL) and 23881 (A375 WT) peptides identified in µPAC LC-MS/MS assay were mapped to potential mutant positions included in a library of MS detectable mutant proteins (corresponding to variants identified by genomics). Some mutated peptides were further successfully validated on SRM.

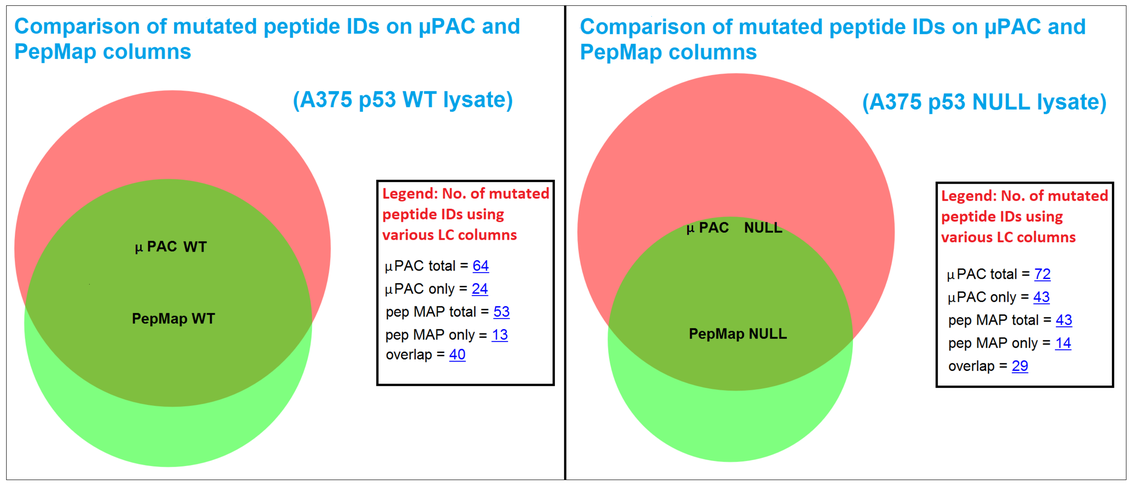

Figure 3. Comparison of mutated peptide IDs on µPAC and PepMap columns in A375 cell line with WT p53 and CRISPR on p53.

Conclusions

The µPAC column showed significantly lower backpressure and higher peak capacity with excellent reproducibility in comparison to the PepMap column. The PepMap column also displayed good peak capacity, although lower than the µPAC, but the retention time's reproducibility was not comparable to the µPAC column. The µPAC showed improved protein IDs compared to PepMap column and also produced higher individual protein sequence coverage. This better sequence coverage is crucial for more readily detecting mutated peptides and eventually neoantigens. After mapping identified peptides to mutant protein sequences we found that the µPAC column was more applicable for detection of mutated peptides possibly because of increased peak capacity. This comparison suggests that our proteogenomic platform will benefit from the better separation of the µPAC column that will provide better coverage of the mutational landscape in tumor tissues.

Novel aspect Pillar-arrayed-column outperforms traditional packed NanoLC column for mutant peptides detection.

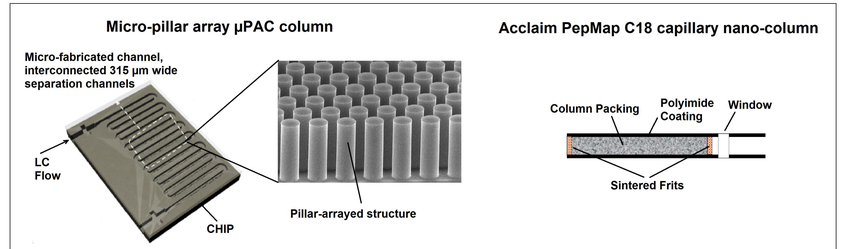

Figure 1. µ-PAC (Pillar-Arrayed-Column (left) and capillary column (right).

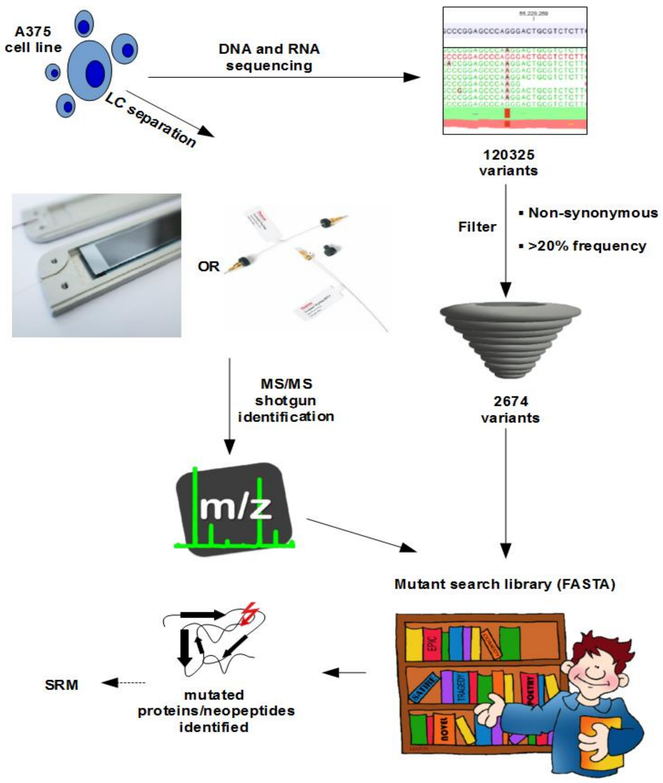

Figure 2. A proteogenomic platform to identify mutations in protein sequences.

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